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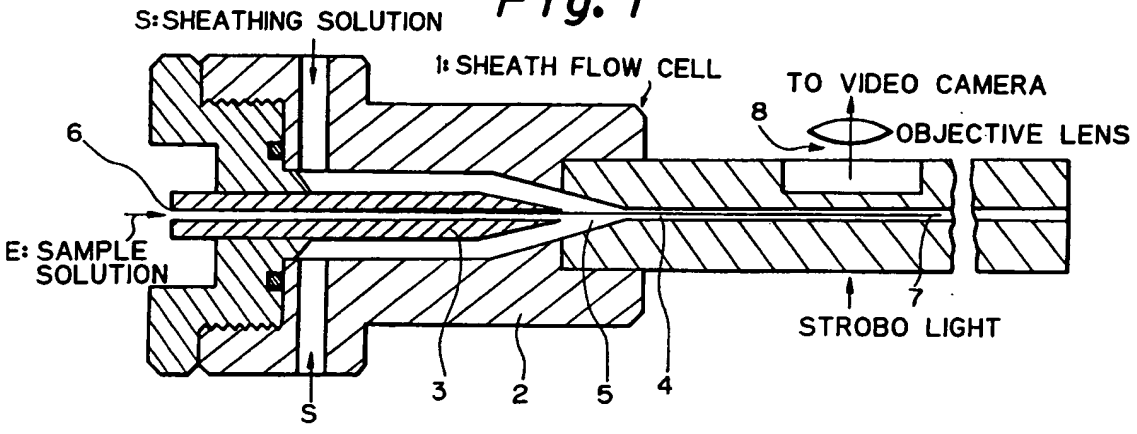
(54) Flow cell mechanism in flow imaging cytometer.

(57) The invention relates to a flow imaging cytometer for imaging the dimensions and shapes of particle components in a flow of a specimen solution has a slender filament (5) serving as a focusing reference provided in a flat imaging zone (8) through which the specimen solution flows in order for the particle components to be imaged. By imaging the filament-shaped focusing reference and adjusting position in accordance with the sharpness of an image of the reference, the particle components in the specimen solution can be brought into sharp focus.

Fig. 1



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This invention r lates to autofocusing in a system in which a specimen such as blood or urine suitably stained is introduced to a flow cell to form a flat, sheathed flow within th cell, the sheathed flow zone is irradiated with strobe light, and a cell image obtained by a video camera is analyzed by application of image processing. More particularly, the invention relates to a flow cell mechanism in a flow imaging cytometer in which a slender filament is passed through a sampling nozzle within the flow cell, the state of focus of an image of the filament is monitored at all times by the apparatus itself and th position of the flow cell or lens is moved finely in automatic fashion when the image of the filament is out of focus, thereby making it possible to readjust focus automatically.

In an image processing system for imaging cells which flow through a flow cell and applying prescribed image processing, an image in which th cells appear at rest by illuminating them with strobe light is obtained. Such an image, which is obtained frame by frame (i.e., every 1/30 of a second), differs from that of the immediately preceding frame. In a still camera or domestic video camera, there is almost no movement of the image from one frame to the next, and therefore focusing of the type in which camera lens is moved in fine increments is comparatively easy to perform. Hower, in a case where the image changes every frame, focus cannot be adjusted by comparing identical images with each other while subjecting the flow cell or lens to fine movement. Even if a focal adjustment in such case is performed manually while viewing the image, it is difficult to judge whether focus is perfect and it takes 5 minutes or more to adjust. Moreover, since the optical system can experience some slippage owing to changes in ambient temperature, re-focusing is required often.

In order to prevent defocusing due to changes in temperature, the apparatus should be controlled so as to hold the overall optical system at a constant temperature. However, such control means raises the cost of the apparatus.

A different expedient is to adopt a method in which the user measures a control fluid, which is employed in order to control the accuracy of the apparatus, after which the apparatus itself performs focusing while finely moving the flow cell or lens based upon the images of particles whose sizes ar uniform within the control fluid. However, in order to correctly obtain an evaluation value indicative of whether or not focusing has been achieved in a case where only a small number of cells appear in a single imaged frame, it is necessary to derive the evaluation value from data indicative of imaged frames consisting of several dozen frames. Consequently, consid rable time is requir d to perform an adjustment while comparing evaluation values from point to point during th fin movement of the flow cell or lens. In addition, this m thod necessitat s not only the reagent but also labor on the part of the user, who is required to measure the control fluid.

An object of the present invention is to provide a novel flow cell mechanism, as well as a focal-point adjustment method in cytometry, which solves the aforementioned problems encountered in the prior art.

According to the present invention, the foregoing object is attained by providing a flow cell mechanism in a flow imaging cytometer of the type in which a specimen solution containing particle components such as cells is made to flow, while sheathed by a sheathing liquid, through an imaging zone of a flat flow path within a flow cell, a still image of the specimen solution flow is photographed in the imaging zone by light irradiating means and imaging means so arranged that an optic axis thereof intersects the flow path, and the still image is subjected to image processing, whereby analysis such as classification and enumeration of the particle components contained in the specimen solution is performed, characterized in that a focusing reference for focus adjustment is provided in the zone through which the specimen solution flows and is photographed.

Further, the foregoing object is attained by providing an automatic focal-point adjustment method in flow imaging cytometry of the type in which a specimen solution containing particle components such as cells is formed into a flat, laminar flow sheathed by a sheathing liquid, a still image of the specimen solution flow is photographed in a flow zone and the still image is subjected to image processing, whereby analysis such as classification and enumeration of the particle components contained in the specimen solution is performed, characterized by imaging a filament-shaped body as a focusing reference for focus adjustment provided in the flow zone, and adjusting position in accordance with clarity of an image of the filament-shaped body, thereby performing a focus adjustment with regard to the particle components in the specimen solution.

Other features and advantages of the present invention will be apparent from the following description taken in conjunction with the accompanying drawings.

Fig. 1 is a longitudinal sectional view illustrating an embodiment of a flow cell mechanism in a cytometer according to the pres nt invention; and

Fig. 2 is a diagram showing an example of an image in which both a filament and a cell particl contained in a sp cimen soluti n resid .

An embodiment of the present invention will

now be described in detail with reference to the accompanying drawings.

As shown in Figs. 1 and 2, a sheath flow cell 1 has a cell body 2 accommodating a sampling nozzle 3 the rear end of which has a fixing portion 6 for fixing the rear end of a slender filament 5 having a free end 7 passed through the interior of the nozzle 3, the diameter whereof is on the order of several microns (e.g., 2 - 5 μm). A sheathing solution S is admitted into the cell body 2 to sheath a sample solution E that flows through a flat flow path 4. The arrangement is such that the filament 5 will flow along with the particles in the sample solution E as it flows by an imaging zone 8 of a video camera. This makes it possible to always pick up the image of the filament 5 at a position substantially fixed in the imaged frame every 1/30 of a second. In addition, if the filament 5 is in focus, then so will be the image of the cell. Rather than concentrating on the images of cells of an indeterminate number appearing at different positions in the imaged frame from one instant to the next, as is done in the prior art, operation according to the invention is simpler and can be performed in a shorter period of time by evaluating focusing based upon the image of the single, substantially unmoving slender filament 5.

An example of an evaluation as to whether an image is in focus is as described in the specification of Japanese Patent Application No. 2-195934. As applied to this invention, quadratic differential values (referred to as a Sobel values or Laplacian values) of the image data are accumulated over the zone in which the filament 5 appears. The value obtained by such accumulation can be used in making an evaluation of focus. Values can be obtained in real-time (every 1/30 of a second) by using an ordinary real-time 3 × 3 convolution circuit as the image processing circuit employed in making the evaluation. In such case, the focal adjustment would be performed in real-time with regard to the imaged frame every 1/30 of a second while minutely moving the flow cell or lens.

Example of materials usable as the filament 5 are metallic fibers, chemically synthesized fibers, ceramic fibers and the like. In any case, use should be made of a strongly corrosion-resistant, highly rigid material which will not bend.

With the arrangement of the present invention described above, a change in the relative positions of the specimen solution and optical system will result in loss of focus and cause blurring of the particl image. However, since the focusing r ference for focal adjustment (namely the filament 5 in the illustrated preferred embodiment) is provided in the zone through which the specimen solution flows, th particle compon nts in th specim n solution can be restored to sharp focus by bringing the focusing r ference into focus.

If th focusing reference adopted is the filament 5, as described and illustrated above, the reference will flow smoothly along with the specimen solution.

In a case where the filament 5 is passed through the sampling nozzle 1 and disposed in the flat flow path 4 of the flow cell, the center of the specimen stream will drift more stably and the filament 5 therefore will virtually be fixed in position.

The image of the filament 5 for focal adjustment purposes is used in a positional adjustment performed by a focal-point adjusting mechanism in accordance with the clarity of the image.

Thus, a flow imaging cytometer according to the present invention provides the following effects:

(1) The apparatus itself is capable of monitoring the status of focus at all time, and therefore focusing can be performed without the intervention of the user.

(2) It is unnecessary to use special particles of a uniform size and shape for the purpose of adjusting focus. This makes it possible to reduce management cost.

(3) The time required for focus adjustment according to the invention is less than that entailed by the method using the aforementioned special particles for adjustment.

(4) A focused image is obtained in which focusing is stable at all times despite changes in ambient temperature.

As many apparently widely different embodiments of the present invention can be made without departing from the spirit and scope thereof, it is to be understood that the invention is not limited to the specific embodiments thereof except as defined in the appended claims.

Claims

1. In a flow imaging cytometer in which a specimen solution containing particle components such as cells is made to flow, while sheathed by a sheathing liquid, through a flat flow path within a flow cell, a still image of the specimen solution flow is photographed by light irradiating means and imaging means so arranged that an optic axis thereof intersects the flow path, and the still image is subjected to image processing, whereby analysis such as classification and enumeration of the particle components contained in the specimen solution is performed, a flow cell mechanism which includes:

a focusing reference for focus adjustment provided in a zone through which th sp cimen solution flows and is photographed.

2. The flow cell mechanism according to Claim 1, wherein said focusing reference is a filament-shaped body.

3. The flow cell mechanism according to Claim 2, wherein said filament-shaped body is arranged in said flat flow path upon being passed through a sampling nozzle of the flow cell.

4. The flow cell mechanism according to Claim 3, wherein said filament-shaped body has a diameter of 2 - 5 μm.

5. The flow cell mechanism according to any one of Claims 1 through 4, further comprising focal-point adjusting means for adjusting position in correlation with sharpness of the image of said focusing reference when said focusing reference is imaged.

6. In flow imaging cytometry in which a specimen solution containing particle components such as cells is formed into a flat, laminar flow sheathed by a sheathing liquid, a still image of the specimen solution flow is photographed in a flow zone and the still image is subjected to image processing, whereby analysis such as classification and enumeration of the particle components contained in the specimen solution is performed, an automatic focal-point adjustment method comprising the steps of:

imaging a filament-shaped body as a focusing reference for focus adjustment provided in the flow zone; and

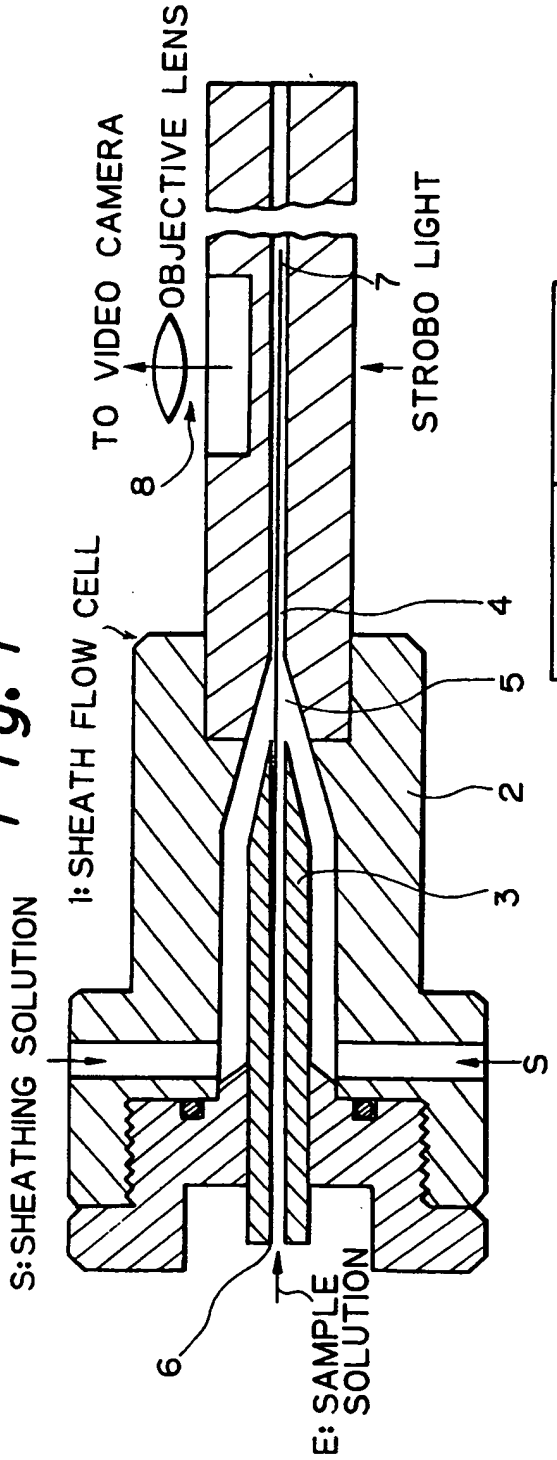
adjusting position in accordance with sharpness of an image of said filament-shaped body, thereby performing a focus adjustment with regard to the particle components in the specimen solution.

7. The focal-point adjustment method according to Claim 6, further comprising a step of continuously imaging said filament-shaped body while finely moving the flow cell or a lens system.

8. The focal-point adjustment method according to Claim 6 or 7, further comprising the steps of:

constantly monitoring state of focus in accordance with said sharpness of the image; and

adjusting position when the state of focus is indicative of a value less than a predetermined value.


TO VIDEO CAMERA
OBJECTIVE LENS
STROBO LIGHT
8
7
4
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2
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S: SHEATHING SOLUTION
1: SHEATH FLOW CELL
E: SAMPLE SOLUTION

Fig. 1

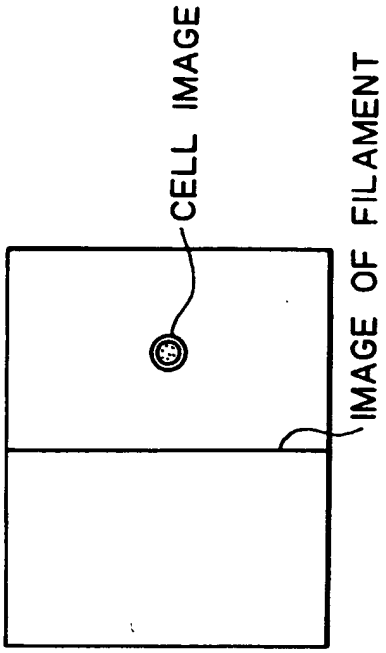
CELL IMAGE
IMAGE OF FILAMENT

Fig. 2

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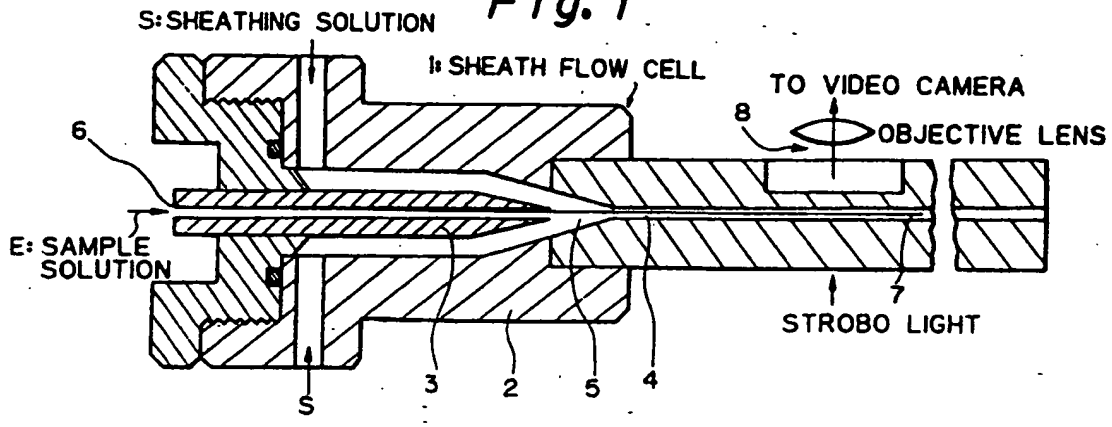
Fig. 1



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EUROPEAN SEARCH REPORT

Application Number

EP 91 10 4764

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
Y	WO-A-8 103 224 (INTERNATIONAL REMOTE IMAGING SYSTEMS) * page 2, line 15 - page 5, line 9 * * abstract; figure 1 *	1	G01N21/05 G01N15/14
Y	PATENT ABSTRACTS OF JAPAN vol. 11, no. 328 (P-629)(2775) 27 October 1987 & JP-A-62 112 034 (CANON) 23 May 1987 * abstract *	1	
A	PATENT ABSTRACTS OF JAPAN vol. 12, no. 259 (P-733)(3106) 21 July 1988 & JP-A-63 047 633 (CANON) 29 February 1988 * abstract *	1	
A	US-A-4 715 708 (ITO) * claim 1; figure 1A *	1	
A	PATENT ABSTRACTS OF JAPAN vol. 11, no. 162 (P-579)(2609) 26 May 1987 & JP-A-61 294 333 (CANON) 25 December 1986 * abstract *	1	
			TECHNICAL FIELDS SEARCHED (Int. Cl.5) G01N

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	21 AUGUST 1992	KRAMETZ E.M.

CATEGORY OF CITED DOCUMENTS

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